

## Influence of Soil Fumigation of Telone and Nemagon on the Ultrastructure of Chromoplasts in Carrot Roots (*Daucus carota* L.)

It has been demonstrated that preplanting soil fumigations of Telone (a mixture of 1,3-dichloropropene and some other chlorinated hydrocarbons) and Nemagon (1,2-dibromo-3-chloropropene) caused significant increases in  $\beta$ -carotene and total carotene in carrot roots<sup>1</sup>. Chromoplasts are the carotenoid containing plastids responsible for yellow, orange or red color of many fruits, flower petals, and certain roots. The carotenoid concentration in the phloem of carrot root was reported to be higher than that in the xylem<sup>2</sup>. The purpose of this study was to investigate effects of soil fumigations of Telone and Nemagon on the ultrastructure of chromoplasts in carrot roots.

**Material and methods.** Carrot seeds *Daucus carota* L. (cultivar Royal Chantenay, J. Harris Co., Rochester, N.Y.) were sown on May, 1969 on a silt loam soil at the Utah State University farm, North Logan, Utah. Telone,

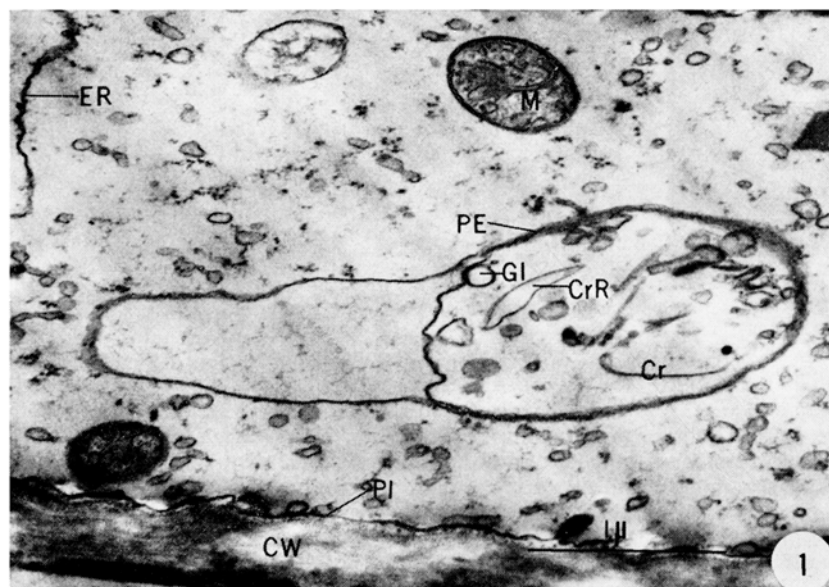
at the rate of 30 gal/acre and Nemagon at the rate of 3 gal/acre were applied by means of a soil fumigant injector 1 week before planting. The soil was irrigated as needed. The roots of uniform size and maturity were selected from each treatment and from the untreated control plots 90 days after planting.

For the electron microscopic studies, transectional slices of phloem of carrot roots approximately 1 mm<sup>3</sup>, were fixed by KARNOVSKY's fixation<sup>3</sup> and 3% OsO<sub>4</sub>. After fixation, tissue were washed with 0.1 M, phosphate buffer pH 7.3, then dehydrated in graded ethanol and

<sup>1</sup> M. WU, B. SINGH, M. T. WU, D. K. SALUNKHE and G. G. DULL, Hort. Sci. 5, 221 (1970).

<sup>2</sup> V. H. BOOTH, J. Sci. Food Agric. 2, 350 (1951).

<sup>3</sup> M. J. KARNOVSKY, J. Cell Biol. 27, 137 (1965).



Figs. 1-3. Electron micrographs of cells in the phloem of carrot roots. Cr, pigment crystalloid; CrR, pigment crystalloid remnant; CW, cell wall; ER, endoplasmic reticulum; GI, globule; M, mitochondrion; PE, plastic envelope; PI, plasmalemma; T, tonoplast.  $\times 28,600$ .

Fig. 1. Carrot root grown in non-fumigated soil.

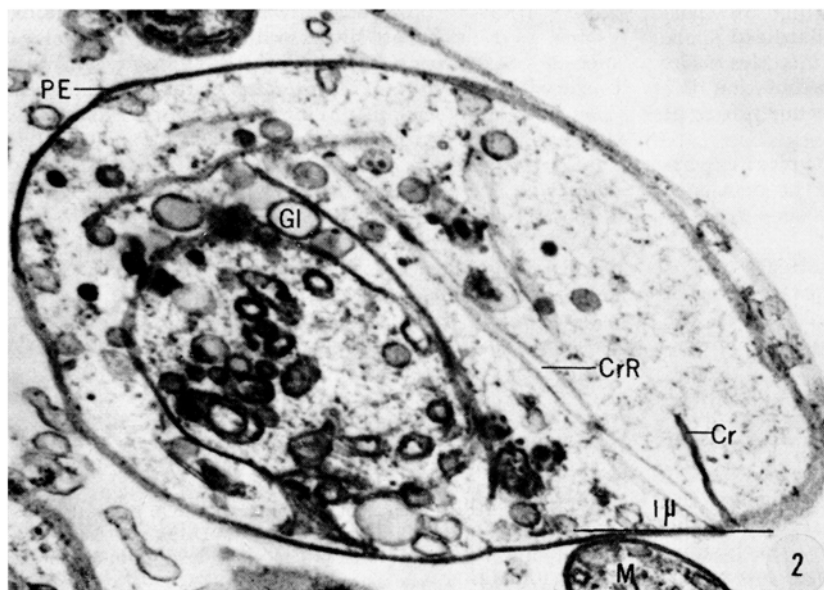


Fig. 2. Carrot root grown in Telone fumigated soil (30 gal/acre).

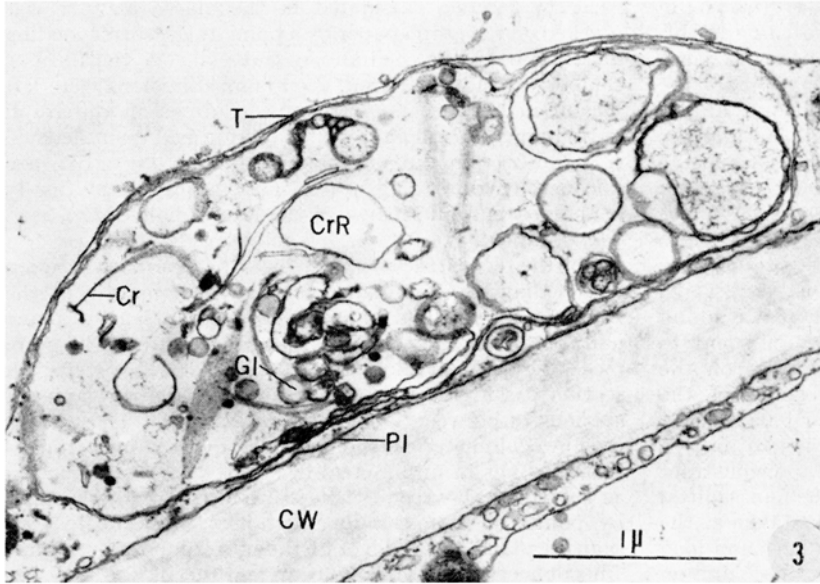


Fig. 3. Carrot root grown in Nemagon fumigated soil (3 gal/acre).

embedded in Epon 812<sup>4</sup>. The blocks were sectioned with a glass knife on a Sorvall Porter-Blum MT-2 ultramicrotome. Sections around 80  $\mu$ m were mounted on the 200 mesh copper grids and stained with uranyl acetate and Reynold's lead, finally examined with a Zeiss EM-9A electron microscope.

**Results and discussion.** The preplanting soil fumigation with Telone and Nemagon significantly influenced the chromoplasts of carrot roots when compared with those from the non-fumigated plots (Figures 1-3), especially the size, shape, and organization of the chromoplasts. The chromoplasts of the carrots grown on the fumigated soil were larger and contained more globules and crystals than those grown on the non-fumigated soil. The chromoplasts of the carrot roots from Telone fumigated soil contained long needle crystals while those of the Nemagon fumigated contained short needle ones. It has been reported that the carotenes of carrot roots were located in the crystals and globules of chromoplasts<sup>5</sup>. It is possible that the increase in amount and size of crystals and globules might result in the increase in carotene content

of the carrot roots. Evidence from the electron microscopic examinations substantiates our previous finding that the soil fumigation with Telone and Nemagon increased total carotenes and  $\beta$ -carotene of carrot roots<sup>6</sup>.

**Zusammenfassung.** Wenn der Boden vor der Aussaat von *Daucus carota* L. mit Telon und Nemagon behandelt wurde, konnten Strukturveränderungen in den Chromoplasten der Karottenwurzeln festgestellt werden.

M. WU and D. K. SALUNKHE

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<sup>4</sup> J. H. LUFT and R. L. WOOD, J. Ultrastruct. Res. 12, 22 (1965).

<sup>5</sup> K. STEFFEN and G. RECK, Planta 60, 627 (1964).

<sup>6</sup> This study was supported by the Agricultural Research Service, U.S.D.A. Grant No. 12-14-100-9903 (61), administered by the Human Nutrition Division, Beltsville, Maryland.

## PRO EXPERIMENTIS

### A Simple Method for Reconstructing Serial Sections

Reconstruction of serial sections is an important and sometimes indispensable step in studying the topography of various organ-systems and organs of the body of an animal. A number of methods for such reconstructions have been described by PETER<sup>1</sup>. The more important of these are the wax model reconstruction method of BORN<sup>2-4</sup> using wax sheets, the graphic reconstruction method of KASTSCHENKO<sup>5</sup> employing squared paper, the graphic reconstruction method of KERR<sup>6</sup> using glass plates immersed in cedarwood oil, and LEWIS's modification<sup>7</sup> of BORN's method by substituting plaster of Paris for wax. DE BEER<sup>8</sup> used blotting paper soaked in wax and PUSEY<sup>9</sup> employed greaseproof 'Sketching Bank' paper immersed in xylol. Most of these methods are, however, tedious and necessitate the sketching of a large number of drawings. The author, while working

on the development of latero-sensory canals and related dermal bones of the head in fishes, found that a simpler method could be equally useful. It is described below with the aid of a specific example.

A millimeter (Graph) paper was taken. The head of an 11 mm long larva of *Ophicephalus punctatus* (a teleostean fish) was cut in serial transverse sections of 12  $\mu$ m thickness. The total number of sections obtained was 225. Since each section was of 12  $\mu$ m or 0.012 mm thickness, the total length of the head to be reconstructed was  $225 \times 0.012 = 2.7$  mm. Since it was considered desirable to plot a magnified reconstruction of this 2.7 mm long head on the graph paper, it was arbitrarily decided that the thickness of 1 section (which, in actual fact, was 0.012 mm) be deemed equivalent to 0.5 mm, and hence, be represented by  $\frac{1}{2}$  of a 1 mm<sup>2</sup> division of the graph paper.